ORIGINAL ARTICLE

Clinical and treatment characteristics determining therapeutic outcome in patients undergoing autologous non-cultured outer root sheath hair follicle cell suspension for treatment of stable vitiligo

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Abstract

Background Autologous non-cultured outer root sheath hair follicle cell suspension (NCORSHFS) is a recently described novel cellular graft technique for the treatment of stable vitiligo. There is lack of data about various factors determining the repigmentation rate in vitiligo patients undergoing this novel surgical therapy.

Objective To study the clinical characteristics and treatment variables determining therapeutic outcome in patients of stable vitiligo undergoing NCORSHFS.

Methods Non-cultured outer root sheath hair follicle cell suspension was prepared from anagen hairs extracted from the occipital area. The number of melanocytes and hair follicle stem cells (HFSC) in the suspension was quantified by staining with anti-HMB45 and anti-CD200 antibody, respectively. In all patients, a 2 mm punch skin biopsy was taken from one of the vitiligo patch to be treated prior to surgery for assessment of histomorphological features. Post surgery patients were followed up at regular intervals for 24 weeks.

Results Thirty patients (21 females, 9 males) with a clinical diagnosis of stable vitiligo, with a total of 60 target lesions were included in this study. The mean age of the study population was 21.10 ± 5.64 years. The number of melanocytes ($P = 0.04$) and HFSC ($P = 0.01$) transplanted were significantly higher among patients achieving optimum repigmentation (>75% repigmentation). There was a strong correlation between repigmentation at 24 week and number of melanocytes and HFSC transplanted. Number of HFSC transplanted and absence of dermal inflammation were significant predictors of achieving optimum repigmentation.

Conclusion The number of melanocytes and HFSC transplanted and absence of dermal inflammation were important determents of optimal repigmentation in patients undergoing NCORSHFS for treatment of stable vitiligo. Hence, refining the technique of NCORSHFS on the basis of these factors would help in achieving better surgical outcomes.

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Conflicts of interest

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Introduction

Vitiligo is a common, often heritable, acquired disorder of melanogenesis. The prevalence of vitiligo is 1%, ranging from 0.1 to more than 8.8% in different parts of the world. The highest incidence has been recorded in inhabitants of the Indian subcontinent.[1] Vitiligo is disfiguring in all races but particularly more so in dark skinned people because of strong contrast.[2] The stigma and social isolation associated with the disease calls for an aggressive treatment.

Though medical management is the first line treatment modality, they generally do not result in complete 'cure' of the disease and residual lesions require surgical treatment. High repigmentation rate are obtained with most of the surgical procedures so far described with variable qualitative outcomes.[3]

Despite the limitations and some side-effects, surgical modalities for vitiligo have evolved tremendously in the last decade and are indicated for all types of stable vitiligo that do not respond to medical therapy.

Surgical modalities for vitiligo can be broadly classified in to tissue grafts and cellular grafts. Autologous non-cultured outer root sheath hair follicle cell suspension (NCORSHFS) is a new novel cellular graft technique for the treatment of vitiligo. We conducted this study to assess the disease and treatment parameters that are associated with optimum repigmentation, (defined as repigmentation of more than 75% at 24 week) in patients undergoing NCORSHFS technique for treatment of stable vitiligo.

Material and methods

Patients

This was an open label prospective study in a tertiary care centre. The study was approved by the 'Institute Ethics Committee' of the Post Graduate Institute of Medical Education and Research, Chandigarh, India. All patients signed an informed consent sheet before inclusion in the study. Thirty patients of stable vitiligo, defined as no fresh lesions and previous lesions not increasing in size for the last 12 months were recruited. The exclusion criteria were actively spreading disease, keloidal tendency, vitiligo patch of >100 cm^2 and patients with unrealistic expectations.

Technique

All patients recruited for the study underwent NCORSHFS technique of cellular grafting. The method of cellular suspension preparation and surgical technique has been described elsewhere.[4,5] For the analysis of the transplanted cell fraction for the presence of melanocytes and hair follicle stem cells (HFSC), a small aliquot (approximately 0.1 million cells or more) of cell pellet was stored at 4°C. The stored cells were used to analysis by immunofluorescence (IF) to find out the percent of HFSC and the differentiated melanocytes. Staining with anti-HMB45 antibody (Abcam, Mouse IgG1, κ Chain) was used as a proxy indicator to assess the number of melanocytes in the cell suspension and staining with anti-CD200 antibody (BD Pharmingen™, Franklin Lakes, NJ, USA, Rat IgG2a, κ Chain, PE conjugated) was used as a marker for HFSC. For the study purpose, all HMB45 staining cell fractions were considered as melanocytes (Fig. 1a) and all CD200 staining cell fractions were considered as HFSC (Fig. 1b).

In all patients, a 2 mm punch skin biopsy was taken from one of the vitiligo patch to be treated prior to surgery. These skin biopsies were subjected to histopathological study with haematoxylin and eosin (H&E) staining for assessment of histomorphology.

Follow-up

The patients were followed up at the clinic on 8th day and 4, 8, 16 and 24 week after the transplantation procedure. No other treatment, other than sun exposure was prescribed to the patients throughout the study period. During every visit, repigmentation was assessed as minimal repigmentation (≤25%), mild repigmentation (26–50%), moderate repigmentation (51–75%), marked repigmentation (76–90%) and excellent repigmentation (90–100%). For the study purpose optimum repigmentation was defined as a repigmentation rate of more than 75% (RP > 75%). Colour matching with the surrounding skin was assessed at 24 week as, same as surrounding skin or somewhat darker/lighter than the surrounding skin.

Statistical methods

The statistical analysis was carried out using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, version 16.0 for Windows). All quantitative variables were expressed using measures of central tendency (mean, median) and measures of dispersion (standard deviation). Normality of data was checked by

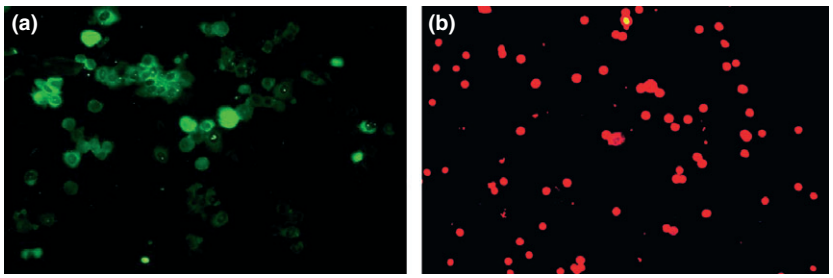

Figure 1 Photomicrographs of immunofluorescence staining of cells in autologous non-cultured outer root sheath hair follicle cell suspension. (a) This photomicrograph shows cells stained with FITC labelled anti-HMB45, with granular fluorescence in the cytoplasm and cell membrane. Cells are oval and large measuring around 7–8 μm in diameter suggestive of melanocytes (IF × 40X). (b) This photomicrograph shows cells stained with PE labelled anti-CD200 with red fluorescence. The cells appear small and round suggestive of hair follicle stem cells (IF × 40X).

Kolmogorov Smirnov test. For normally distributed data, means were compared using student's *t*-test for outcome. For skewed data or scores Mann–Whitney test was used. Qualitative or categorical variables were described as frequencies and proportions. Proportions were compared using Chi square or Fisher's exact test whichever was applicable. Predictors of clinical response were assessed using logistic regression analysis. All statistical tests were two-sided and performed at a significance level of $P < 0.05$.

Results

Patient characteristics

Thirty patients with a clinical diagnosis of stable vitiligo, having a total of 60 target lesions were included in this study. Twenty one patients were females and nine were males. The mean age of the study population was 21.10 ± 5.64 years (range 8–38 years). In the study population, vitiligo was present for a mean duration of 6.90 ± 3.92 years and the stability period was 3.62 ± 2.37 years. Seventeen patients had focal vitiligo, 11 patients had generalized vitiligo and 2 patients had segmental vitiligo. The lesions treated surgically in this study were widely distributed all over the body. Thirty-one (51.67%) lesions were present over proximal extremities (leg, thigh and arm), 18 (30%) lesions over acral areas (feet and hand), 8 (13.33%) over head and neck area and 3 (5%) over trunk. All patients had previously received various medical treatments for management of their condition, including topical corticosteroids, topical calcineurin inhibitors, PUVA, PUVASOL and NBUVB. These patients had either not responded to the medical treatment modalities, or had partial response with few recalcitrant lesions remaining resistant to therapy. Pretreatment skin biopsy was done in 29 patients prior to surgical therapy. On H&E staining of the pretreatment tissue samples, pigmentation and basal melanocytes were uniformly absent in all the tissue sections. Other epidermal changes noted were hyperkeratosis (5, 17.24%), acanthosis (3, 10.35%) and flattening of rete pegs (5, 17.24%). Basal cell vacuolization was seen in two patients. No significant dermal pathology was noted in 11 patients (37.93%). Upper dermal fibrosis was seen in 12 (41.38%) patients (Fig. 2a) and 8 (27.58%) patients had dermal inflammation (Fig. 2b). The number of melanocytes transplanted ranged from 96 to 2747 cells/cm^2 and the number of HFSC transplanted ranged from 81 to 1442 cells/cm^2. This was done to compare the efficacy of repigmentation with different concentrations of the various cell fractions. All 30 patients completed the study period of 24 weeks and were included in the final analysis.

75% repigmentation

Optimum repigmentation (RP > 75%) was seen in 21 of 60 (35%) lesions (Table 1). Ten of these 21 lesions had achieved excellent repigmentation defined as repigmentation rate of more than 90% (RP > 90%) (Fig. 3). Ten (32.3%) lesions over proximal extremity, 4 (22.2%) lesions over acral sites, 5 (62.5%) lesions over head and neck area and 2 (66.7%) lesions over trunk achieved RP > 75% (Table 2). There was no statistically significant difference in the repigmentation rate among the different body sites ($P = 0.146$). Depending on the type of vitiligo 13 (40.6%) lesions of focal vitiligo, 6 (24%) lesions of generalized vitiligo and 2 (66.7%) lesions of segmental vitiligo achieved RP > 75% (Table 3). There was no statistically significant difference in the repigmentation rate among different types of vitiligo ($P = 0.21$).

On univariate analysis, there was a statistically significant difference in the number of melanocytes and HFSC transplanted per unit of treated area between patients achieving RP > 75% and not achieving RP > 75%. The mean number of melanocytes transplanted in patients achieving RP > 75% was 1187 cells/cm^2 compared to 796 cells/cm^2 in patients not achieving RP > 75% (Table 1, $P = 0.04$). The mean number of HFSC transplanted in patients achieving RP > 75% was 756 cells/cm^2 compared to 494 cells/cm^2 in patients not achieving RP > 75% (Table 1,

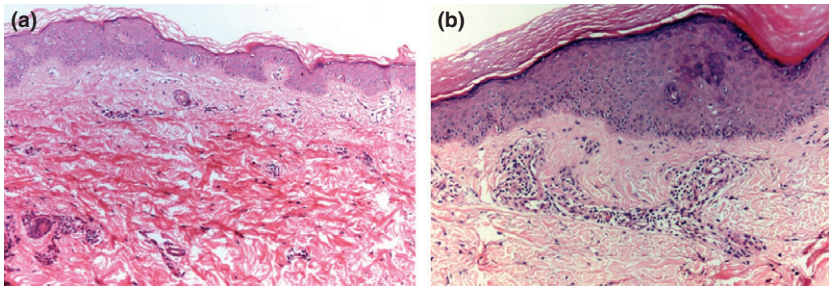

Figure 2 Photomicrographs of histopathological examination of pretreatment skin biopsies. (a) This photomicrograph shows complete absence of epidermal pigmentation and thickened collagen bundles with minimal perivascular inflammation in upper dermis (H&E × 10X). (b) This photomicrograph from dorsum of feet shows hyperkeratosis, acanthosis, complete absence of epidermal pigmentation and moderate perivascular inflammation in upper dermis (H&E × 10X).

Table 1 Comparison of clinical characteristics and treatment variables between patients achieving and not achieving repigmentation of more than 75%

Characteristics	Repigmentation rate		*P* value
	>75%	≤75%	
Number of lesions	21	39	
Age (years)	23.57 ± 8.58	21.51 ± 3.79	0.31
Total disease duration (years)	7.29 ± 4.00	6.87 ± 3.81	0.7
Stability (years)	3.57 ± 1.96	3.56 ± 2.36	0.99
Melanocytes/cm^2	1187 ± 711	796 ± 649	0.04*
Hair follicle stem cells/cm^2	756 ± 384	494 ± 357	0.01*

*Significant *P* value.

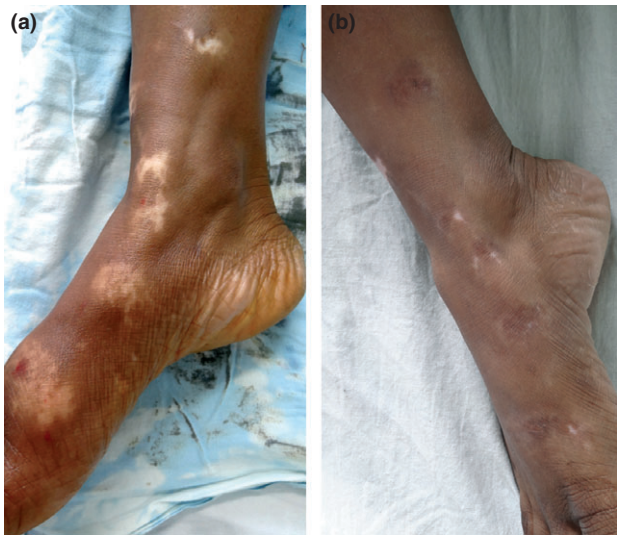

Figure 3 (a) Pretreatment photograph of focal vitiligo over acral sites. (b) Post-treatment photograph showing optimum (>90%) repigmentation with good colour matching. The number of melanocytes and hair follicle stem cells (HFSC) transplanted were 1600 and 835 cells/cm^2, respectively.

P = 0.01). However, there was no significant difference in terms of age, gender, total disease duration, stability of lesion, site of lesion and past history of phototherapy between patients achieving RP > 75% and not achieving RP > 75%. On histopathological analysis, inflammation was significantly more common in patients not achieving RP > 75% (*P* = 0.023). None of the other histological changes showed any significant correlation.

90% repigmentation

Repigmentation rate of more than 90% (RP > 90%) was seen in 10 of 60 (16.66%) lesions (Table 4). Seven (22.6%) lesions over proximal extremity, 1 (5.6%) lesion over acral site and 2 (25%) lesions over head and neck area achieved RP > 90% (Table 2). However, there was no statistically significant difference in the repigmentation rate among the different body sites (*P* = 0.337). Depending on the type of vitiligo 8 (25%) lesions of focal vitiligo, 1 (4%) lesion of generalized vitiligo and 1 (33.3%) lesion of segmental vitiligo achieved RP > 90% (Table 3). There was no statistically significant difference in the repigmentation rate among different types of vitiligo (*P* = 0.07).

On univariate analysis, there was a statistically significant difference in the number of HFSC transplanted per unit of treated area between patients achieving RP > 90% and not achieving RP > 90%. The mean number of HFSC transplanted in patients achieving RP > 90% was 871 cells/cm^2 compared to 529 cells/cm^2 in patients not achieving RP > 90% (Table 4, *P* = 0.01). There was no significant difference in terms of age, gender, total disease duration, stability of lesion, site of lesion, past history of phototherapy and number of melanocytes transplanted per unit area between patients achieving RP > 90% and not achieving RP > 90%. On histopathological analysis, inflammation was significantly more common in patients not achieving RP > 90% (*P* = 0.05). None of the other histological changes showed any significant correlation.

Predictors of optimum repigmentation

On logistic regression analysis, the variables that significantly predicted optimum repigmentation (RP > 75%) were HFSC

Table 2 Distribution of lesions and repigmentation

Sites	>75% repigmentation at 24 week*		>90% repigmentation at 24 week#		Total
	Yes (%)	No (%)	Yes (%)	No (%)	
Proximal extremity	10 (32.3)	21 (67.7)	7 (22.6)	24 (77.4)	31
Acral	4 (22.2)	14 (77.8)	1 (5.6)	17 (94.4)	18
Face and neck	5 (62.5)	3 (37.5)	2 (25)	6 (75)	8
Trunk	2 (66.7)	1 (33.3)	0 (0)	3 (100)	3
Total	21	39	10	50	60

**P* value, 0.146.
#*P* value, 0.337.

Table 3 Type of vitiligo and optimum repigmentation

Sites	>75% repigmentation at 24 week*		>90% repigmentation at 24 week#		Total
	Yes (%)	No (%)	Yes (%)	No (%)	
Focal vitiligo	13 (40.6)	19 (59.4)	8 (25)	24 (75)	32
Generalized vitiligo	6 (24)	19 (76)	1 (4)	24 (96)	25
Segmental vitiligo	2 (66.7)	1 (33.3)	1 (33.3)	2 (66.7)	3
Total	21	39	10	50	60

*P value, 0.21.
#P value, 0.08.

Table 4 Comparison of clinical characteristics and treatment variables between patients achieving and not achieving repigmentation of more than 90%

Characteristics	Repigmentation rate		*P* value
	>90%	≤90%	
Number of lesions	10	50	
Age (years)	26.30 ± 11.28	21.4 ± 23.87	0.21
Total disease duration (years)	7.30 ± 3.13	6.96 ± 4.00	0.80
Stability(years)	4.3 ± 1.70	3.42 ± 2.28	0.25
Melanocytes/cm^2	1293 ± 523	861 ± 702	0.07
Hair follicle stem cells/cm^2	871 ± 240	529 ± 384	0.01*

*Significant *P* value.

transplanted per unit of treated area ($P = 0.02$) and absence of dermal inflammation ($P = 0.013$, Table 5). The odd's ratio of absence of inflammation predicting RP > 75% was 24.13 (Table 5). The variables that did not predict the outcome include age, gender, stability, previous phototherapy, site of lesion and presence of dermal fibrosis.

Dermal inflammation was found in 8 (27.6%) of the 29 skin biopsies analysed. The mean number of melanocytes and HFSC transplanted in patients with dermal inflammation was 1228.26 ± 978.78 cells/cm^2 and 570.1 ± 424.2 cells/cm^2, respectively. There was no statistically significant difference in the number of melanocytes ($P = 0.32$) or HFSC ($P = 0.63$) transplanted between patients who showed dermal inflammation on skin biopsy and patients in whom dermal inflammation was absent.

Correlation between repigmentation at 24 week and cell fraction's in NCORSHFS

There was a significant positive correlation between repigmentation rate at 24 week and melanocytes ($P = 0.02$, $r = 0.3$) and HFSC ($P = 0.002$, $r = 0.4$) transplanted per unit of treated area. There was also a positive correlation between melanocytes and HFSC transplanted per unit of treated area ($r = 0.29$, $P = 0.023$).

Colour matching

Twenty-six (43.33%) of the treated lesions showed excellent colour matching with the surrounding skin. Twenty-three (38.33%) treated lesions were somewhat darker than the surrounding skin and two (3.33%) lesions were somewhat lighter than the surrounding skin. Nine (15%) lesions did not achieve any pigmentation. There was no statistically significant difference in the number of melanocytes ($P = 0.08$) or HFSC ($P = 0.6$) transplanted between patients who showed hyperpigmentation compared to those who did not.

Discussion

Cellular graft techniques are among the common surgical techniques used in the management of stable vitiligo. NCORSHFS is a recently described novel cellular graft technique for the surgical treatment of stable vitiligo.[4] Hair follicle melanocytes have some unique characteristics that make them an attractive source of melanocytes than epidermis for cell based therapies in vitiligo. In follicular melanin unit, there is higher density of melanocytes and melanocyte stem cells.[6,7] Hair melanocytes have remarkable synthetic capacity and a relatively small number of melanocytes

Table 5 Predictors of more than 75% repigmentation

	B (SE)	95% CI for odd's ratio			Percentage prediction
		Lower	Odds ratio	Upper	
HFSC/cm^2	0.003 (0.01)	1.001	1.003	1.006	82.8%
Absence of dermal inflammation	3.18 (1.28)	1.981	24.129	293.961	
Constant	−5.584 (1.62)		0.004		

B, Odd's ratio; HFSC, hair follicle stem cells; SE, standard error.

can potentially produce sufficient melanin to pigment up to 1.5 m of hair shaft.[8]

Two studies have so far described the use of NCORSHFS in the treatment of vitiligo.[4,5] Mohanty *et al.*[4] in their pioneering study reported the use of NCORSHFS in the treatment of vitiligo. The authors documented a mean repigmentation rate of 65.7% and 9 out of 14 patients (64.2%) achieved >75% repigmentation. The mean repigmentation rate was significantly less in patients with disease stability of less than 12 months. Hence, the authors recommended NCORSHFS only in patients with stable vitiligo for at least 12 months.

In a previous study, we compared the treatment outcome in patients of stable vitiligo treated with NCORSHFS method and epidermal cell suspension method.[5] Excellent repigmentation (90–100%) was seen in 83.3% of the treated lesions in epidermal cell suspension group and 65.2% of the treated lesions in the NCORSHFS group. However, there was no statistically significant difference in the treatment outcome between both the groups. Hence, both epidermal cell suspension method and NCORSHFS method was found to be of comparable efficacy in treating patients of stable vitiligo. However, neither of the previous studies assessed the disease characteristics and treatment variables which are associated with better repigmentation.

The current study included 30 patients with 60 lesions of stable vitiligo. The mean age of our study population was 21 years with a preponderance of females. This is probably the result of greater cosmetic awareness and social stigma of the disease in young, unmarried females.[9] The current consensus recommends the disease stability of at least 12 months for the purpose of surgical treatment of vitiligo.[10] However, the concept of stability is ever changing and at present individual lesional stability rather than global stability of 12 months is considered optimal.[11] Hence, for ethical reasons only patients with a disease stability of at least 12 months were recruited in the present study.

Twenty-five percent of lesions of focal vitiligo and 33.3% of lesion of segmental vitiligo achieved RP > 90% compared to 4% of generalized vitiligo. This difference in the repigmentation rate tended to be significant ($P = 0.07$). Similar findings were reported by Mohanty *et al.*[4] where all the patients who failed treatment had vitiligo vulgaris. Previous studies of epidermal cell suspension technique have also reported similar findings with better repigmentation achieved by patients with segmental and focal vitiligo.[12,13]

Tegta *et al.*[14] in a study done on epidermal cell suspension transplantation for treatment of stable vitiligo found that >75% repigmentation was achieved by 50% of the patients, when treated with a mean melanocyte density of 2316 ± 270 cells/cm^2. Hence, the authors concluded that the minimum number of melanocytes in epidermal cell suspension required to produce satisfactory repigmentation (>75%) was probably in the range of 2100–2500 cells/cm^2.[14] Currently, there is no data on the number of melanocytes that is required to be transplanted in NCORSHFS for optimal repigmentation. Mohanty *et al.* transplanted a mean number of 36 285 ± 9659 cells in their series. However, the authors had not standardized the amount of cells that were transplanted. Based on the studies conducted in epidermal cell suspension, the authors had speculated the cell requirement to be less than 2000 cells/cm^2.[4,14] In the present study, we found a significant correlation between the number of melanocytes transplanted and repigmentation rate at 24 week. When assessing the clinical and treatment characteristics associated with optimal pigmentation, we found that a statistically significant higher melanocytes were transplanted in patients achieving RP > 75% (Table 1). In our study the mean number of melanocytes transplanted that was associated with optimum pigmentation was 1187 cells/cm^2, which is much less than that described by Tegta *et al.*[14] This lesser requirement of melanocyte for optimal pigmentation than epidermal cell suspension may be due to the different morphology and ultrastructural characteristics of hair follicle melanocytes, being larger, more dendritic and a higher synthetic capacity.[8] However, on assessing the clinical and treatment characteristics in patients achieving RP > 90% and patients not achieving RP > 90% there was no significant difference in the number of melanocytes transplanted. This suggests that after a certain melanocyte concentration, repigmentation achieved reaches a plateau. This was also evident in the study by Hong *et al.*[15] where the authors used two high melanocyte concentrations, 7970 ± 1560 cells/cm^2 and 7110 ± 1150 cells/cm^2 and found no difference in the repigmentation rate.

Hair follicle is an important reservoir of melanocytes and their precursor cells. Melanocyte-lineage antigens plus c-Kit (the receptor for stem cell factor) stained cells are localized in the outer layer of the outer root sheath of the infundibulum and mid-follicle and the matrix of the hair bulb.[16] This reservoir of melanocytes and melanocytes stem cells is important in the treatment of vitiligo as the initial repigmentation in vitiligo patches often occurs around the hair follicles and vitiligo patches on skin lacking hair follicles, such as palms and eyelids, are often resistant to medical therapies.[17] In our study, we found a significant correlation between the number of HFSC transplanted and repigmentation rate at 24 week. In addition, we found a significant difference in the number of HFSC transplanted both in the groups achieving RP > 75% and RP > 90% compared to group not achieving these end points. These observations suggest that the transplanted HFSC have a significant influence on the extent of repigmentation achieved.

Interestingly colour mismatch with hyperpigmentation of the treated area was found in a significant number of cases in our study. The mean number of melanocytes transplanted was higher (1163 vs. 791 cells/cm^2) in patients showing hyperpigmentation compared to others, though it was not statistically significant ($P = 0.07$). This might be due to the remarkable synthetic capacity of the hair follicle melanocytes as discussed

previously.[8] Another significant finding associated with poor repigmentation in our study population was presence of dermal inflammation. There was a statistically significant higher number of patients who showed dermal inflammation among groups not achieving RP > 75% and RP > 90%. Furthermore, absence of dermal inflammation was one of the predictors of RP > 75%. Rao *et al.*[18] studied the clinical, biochemical and immunological factors determining stability of disease in patients with generalized vitiligo undergoing melanocyte transplantation. The authors found that a higher percentage of CD8 and CD45RO cells in lesional biopsy was associated with failure of repigmentation after transplantation. These findings suggest that presence of an inflammatory milieu at the recipient site is associated with failure of repigmentation probably because of its negative effect on transplanted melanocytes. Apart from some known and well studied clinical parameters like 'stability' the present focus of vitiligo research with respect to treatment outcome is also histopathological/immunohistochemical and serological characteristics as predictors of repigmentation response. However, the role of routinely assessing all patients undergoing vitiligo surgery by skin biopsy is controversial and an area of future research.[11] The limitation of the present study includes a small sample size.

In conclusion, our study suggests that the number of melanocytes and HFSC transplanted are important determents for optimal repigmentation in patients undergoing NCORSHFS for treatment of stable vitiligo. Absence of dermal inflammation on skin biopsy is an important predictor for optimal repigmentation.

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References

1 Sehgal VN, Srivastava G. Vitiligo: compendium of clinico-epidemiological features. *Indian J Dermatol Venereol Leprol* 2007; **73**: 149–156.
2 Mattoo SK, Handa S, Kaur I, Gupta N, Malhotra R. Psychiatric morbidity in vitiligo: prevalence and correlates in India. *J Eur Acad Dermatol Venereol* 2002; **16**: 573–578.
3 Gupta SOM, Kanwar AJ, Ortonne JP. Surgical Management of Vitiligo, 1st edn. Blackwell Publishing Ltd, Oxford, 2007.
4 Mohanty S, Kumar A, Dhawan J, Sreenivas V, Gupta S. Noncultured extracted hair follicle outer root sheath cell suspension for transplantation in vitiligo. *Br J Dermatol* 2011; **164**: 1241–1246.
5 Singh C, Parsad D, Kanwar AJ, Dogra S, Kumar R. Comparison between autologus noncultured extracted hair follicle outer root sheath cell suspension and autologous non cultured epidermal cell suspension in the treatment of stable vitiligo: a randomized study. *Br J Dermatol*. 2013; **169**: 287–293.
6 Tobin DJ, Paus R. Graying: gerontobiology of the hair follicle pigmentary unit. *Exp Gerontol* 2001; **36**: 29–54.
7 Gho CG, Braun JE, Tilli CM, Neumann HA, Ramaekers FC. Human follicular stem cells: their presence in plucked hair and follicular cell culture. *Br J Dermatol* 2004; **150**: 860–868.
8 Legue E, Sequeira I, Nicolas JF. Hair follicle renewal: authentic morphogenesis that depends on a complex progression of stem cell lineages. *Development* 2010; **137**: 569–577.
9 Parsad D, Dogra S, Kanwar AJ. Quality of life in patients with vitiligo. *Health Qual Life Outcomes* 2003; **1**: 58.
10 Parsad D, Gupta S. Standard guidelines of care for vitiligo surgery. *Indian J Dermatol Venereol Leprol* 2008; **74**(Suppl): S37–S45.
11 Ezzedine K, Lim HW, Suzuki T *et al.* Revised classification/nomenclature of vitiligo and related issues: the Vitiligo Global Issues Consensus Conference. *Pigment Cell Melanoma Res* 2012; **25**: E1–E13.
12 Mulekar SV. Long-term follow-up study of segmental and focal vitiligo treated by autologous, noncultured melanocyte-keratinocyte cell transplantation. *Arch Dermatol* 2004; **140**: 1211–1215.
13 van Geel N, Wallaeys E, Goh BK, De Mil M, Lambert J. Long-term results of noncultured epidermal cellular grafting in vitiligo, halo naevi, piebaldism and naevus depigmentosus. *Br J Dermatol* 2010; **163**: 1186–1193.
14 Tegta GR, Parsad D, Majumdar S, Kumar B. Efficacy of autologous transplantation of noncultured epidermal suspension in two different dilutions in the treatment of vitiligo. *Int J Dermatol* 2006; **45**: 106–110.
15 Hong WS, Hu DN, Qian GP, McCormick SA, Xu AE. Ratio of size of recipient and donor areas in treatment of vitiligo by autologous cultured melanocyte transplantation. *Br J Dermatol* 2011; **165**: 520–525.
16 Randall VA, Jenner TJ, Hibberts NA, De Oliveira IO, Vafaee T. Stem cell factor/c-Kit signalling in normal and androgenetic alopecia hair follicles. *J Endocrinol* 2008; **197**: 11–23.
17 Cui J, Shen LY, Wang GC. Role of hair follicles in the repigmentation of vitiligo. *J Invest Dermatol* 1991; **97**: 410–416.
18 Rao A, Gupta S, Dinda AK *et al.* Study of clinical, biochemical and immunological factors determining stability of disease in patients with generalized vitiligo undergoing melanocyte transplantation. *Br J Dermatol* 2012; **166**: 1230–1236.